Kidney International, Vol. 48 (1995), pp. 1559–1562

Diurnal variation in glomerular charge selectivity, urinary albumin excretion and blood pressure in insulin-dependent diabetic patients

PERNILLE M. HANSEN, PATRICIA P.M. GODDIJN, ALLAN KOFOED-ENEVOLDSEN, KARIN M. VAN TOL, HENK J.G. BILO, and TORSTEN DECKERT

Steno Diabetes Center, Gentofte, Denmark, and Hospital De Weezenlanden, Department of Internal Medicine, Zwolle, The Netherlands

Diurnal variation in glomerular charge selectivity, urinary albumin excretion and blood pressure in insulin-dependent diabetic patients. The urinary albumin excretion rate (AER) in a subgroup of patients with insulin-dependent diabetes mellitus (IDDM) steadily increases. In these patients a concomitant reduction of the glomerular charge selectivity index (SI) has been demonstrated. The aim of the present study was to evaluate whether diurnal variation in AER could be related to a diurnal variation in SI and/or a diurnal blood pressure variation. Thirty-three patients with IDDM, 27 with normal albumin excretion (AER <20 μg/min; group D_0) and six with incipient nephropathy (AER from 20 to 200 μg/min; group D_A), were studied. AER and SI (renal clearance ratio of total-IgG/IgG_4) were measured in three different urine collecting periods: period A (8:00 a.m. to 12:00 a.m.), period B (12:00 a.m. to bedtime) and period C (bedtime to 8:00 a.m.). A significant increase in SI was seen during the nighttime: period A, 1.6 (0.2 to 3.8; mean, range); period B, 1.7 (0.3 to 3.0); and period C, 2.0 (0.2 to 4.0); $P = 0.01$. Corresponding to this observation, an overall significant decrease in AER was found: period A, 10 (3 to 137) μg/min (median, range); period B, 8 (3 to 84) μg/min; and period C, 5 (0 to 78) μg/min; $P < 0.001$. In all three sampling periods a negative correlation was found between AER and SI. When group D_0 was analyzed alone, the results were similar. Diurnal variation in blood pressure was significantly positively correlated with AER in group D_A, but was not correlated to variation in AER in D_0. We suggest that in normoalbuminuric IDDM patients diurnal variation in AER is related to diurnal variation in SI.

In patients with insulin-dependent diabetes mellitus (IDDM), the cumulative incidence of diabetic nephropathy is approximately 30 to 40% [1]. In this subgroup of patients, their urinary albumin excretion rate (AER) is steadily increasing. Additional clinical or biochemical markers associated with the very earliest pathophysiological events of diabetic nephropathy are needed to clarify the nature of such a progressive leakiness of the urinary filtration barrier.

So far, several studies have shown that in IDDM albuminuria is associated to reduced amounts of the anionic molecule heparan sulfate proteoglycan (HSPG) located within the extracellular matrix [2–6]. The glomerular charge selectivity index (SI) obtained by estimating the renal clearance of two molecules similarly sized but differently charged, has been suggested to reflect the glomerular charge, determined by the glomerular content of HSPG [7]. It has been demonstrated that SI is significantly reduced in patients with IDDM and albuminuria, even in the subclinical range (AER < 200 μg/min) [8, 9]. Moreover, SI has recently been correlated to other well-known risk markers for albuminuria such as poor metabolic control and glomerular basement membrane thickness [10, 11]. Consequently, the calculated SI might be a candidate marker identifying IDDM patients at risk for developing nephropathy.

However, if SI shall be of any clinical importance, we need to gain further knowledge about the interrelationship between AER and SI in patients with normoalbuminuria or incipient nephropathy. So far a negative correlation between SI and AER in patients with incipient nephropathy has been demonstrated [12]. The aim of the present study was to evaluate whether an expected diurnal variation in AER could be related to a diurnal variation in the glomerular charge selectivity index and/or a variation in systemic blood pressure in patients with IDDM.

Methods

Patients

The study was designed as a cross-sectional cohort evaluation with subgroup analysis, including IDDM patients with or without albuminuria.

The patients were selected from the outpatient clinic at a district hospital in The Netherlands. The protocol was approved by the regional scientific ethical committee and the participants gave informed consent. Forty-seven patients with IDDM were enrolled in the study. They had an onset of diabetes before the age of 40 years and required immediate treatment with insulin. Except from insulin administrated by pens (80% of the patients had at least 4 injections per day) the patients had no other medications. The patients were either normoalbuminuric (AER < 20 μg/min) or had incipient nephropathy (AER in the range of 20 to 200 μg/min). They had no history of non-diabetic renal disease. In two patients urine samples were lost during transportation, and in 12 patients urinary IgG_4 levels were below the detection level of our assay, leaving 33 patients (27 normoalbuminurics in group D_0 and

Received for publication January 12, 1995
and in revised form June 1, 1995
Accepted for publication June 2, 1995

Table 1. Baseline clinical and laboratory data in the total study population ($N = 33$) and in the subgroups D_0 ($N = 27$) and D_A ($N = 6$)

	Sex *M/F*	Age *years*	Diabetes duration *years*	Total-glycohemoglobin *%*	sBP *mm Hg*	dBP *mm Hg*	Creatinine clearance *ml/min*
IDDM (AER < 200 μg/min) ($N = 33$)	18/15	38 (18–64)	15 (2–43)	7.2 (3.2–12.1)	131 (100–168)	80 (58–108)	120 (71–179)
Subgroups							
IDDM (D_0) normoalbuminuric (AER <20 μg/min)	14/13	38 (18–64)	13 (2–42)	7.5[a] (4.2–12.1)	128 (100–168)	79 (58–108)	124 (97–179)
IDDM (D_A) incipient nephropathy (AER 20–200 μg/min)	4/2	40 (24–62)	21 (5–43)	5.5[a] (3.2–6.7)	142 (107–163)	87 (68–96)	101 (71–135)

Data are presented as mean (range).
[a] Normoalbuminuric patients vs patients with incipient nephropathy, $P = 0.01$

6 with incipient nephropathy in group D_A) to be included in the analysis. Further clinical data are given in Table 1.

Measurements

Blood samples including S_{IgG}, S_{IgG4}, total-glycohemoglobin and serum creatinine (S_{Cr}) were taken in the morning. Thereafter ambulatory 24-hour blood pressure measurement (AMBM) and 24-hour urine collection were started. The patient went home doing ordinary daily activities avoiding excessive physical exercise. The 24-hour urine was collected in three sampling periods: morning-period (8:00 a.m. to 12:00 a.m.; period A), afternoon-period (12:00 a.m. to bedtime; period B) and nighttime-period (bedtime to 8:00 a.m.; period C). The subjects recorded their sleeping hours. In each sampling period urinary IgG (U_{IgG}), urinary IgG_4 (U_{IgG4}), AER, urinary creatinine (U_{Cr}), and urinary retinol-binding protein (U_{RBP}) were measured. S_{IgG}, S_{IgG4}, U_{IgG} and U_{IgG4} were measured by ELISA [13]. Urine samples were stored as previously described and assayed within 12 weeks from sampling [13]. Within assay and day-to-day coefficient of variations were below 10%. SI was calculated as $SI = (U_{IgG}/S_{IgG})/(U_{IgG4}/S_{IgG4})$, the subclass IgG_4 being more anionic than total IgG (IgG_4, radius 55Å, isoelectric point 5.5 to 6.0, vs. IgG, radius 55Å, isoelectric point 5.8 to 7.3).

AER was determined using a nephelometric method (Beckman Array analyzer) interassay variation 3.5%, lowest detection level 0.2 mg/liter. The average daytime-AER was calculated as AER [μg/min (period A)] × period A (min) + AER [μg/min (period B)] × period B (min)/[period A (min) + period B (min)], and the percentage change in AER (Δ-AER) as [(average daytime-AER − nighttime-AER)/(average daytime-AER)] × 100.

U_{RBP}, measured by ELISA method, had an interassay variation from 1.2 to 3.1% and a day-to-day coefficient of variation from 9.2 to 10.5% [14]. Total-glycohemoglobin was measured by affinity chromatography (Pierce columns; normal range 4.2 to 5.8%) and serum and urinary creatinines were determined by Jaffe reaction (Hitachi 717 automatic analyzer), and had an interassay variation of 2.5%.

Noninvasive 24-hour ambulatory blood pressure was performed using an automated device (Spacelabs 90207) which was programmed to measure systolic and diastolic blood pressures (sBP and dBP) every 30 minutes from 0800 to 2200, and every 60 minutes from 2200 to 0800. Mean blood pressure (mBP) was calculated. In addition to mean sBP and dBP also the percentage change (Δ-BP) from day to night was calculated as [(average daytime-BP − average nighttime-BP)/(average daytime-BP)] × 100.

Statistics

Results are expressed as mean (range) except from AER and U_{RBP} given as median (range). Differences between groups (D_0 and D_A) were assessed by the unpaired Mann-Whitney test. Within groups, differences in AER, SI, S_{IgG}, S_{IgG4}, U_{IgG}, U_{IgG4} and U_{RBP} in different sampling periods (A, B and C) were assessed by paired Friedmann tests, and day-to-night differences in systolic and diastolic blood pressures were evaluated using the paired Wilcoxon test. Correlations were expressed by Pearson's correlation coefficient, r, AER were logarithmically transformed. P values < 0.05 were accepted as significant. Data were processed using the StatGraphics statistical software package (STSC Inc., Rockville, MD, USA).

Results

The median 24-hour AER in the 33 patients was 8 (2 to 90) μg/min (median, range): 6 (2 to 19) μg/min and 69 (27 to 90) μg/min in groups D_0 and D_A, respectively. Apart from total glycohemoglobin, mean age, diabetes duration, sBP, dBP and clearance of creatinine were comparable between groups (Table 1).

During the 24-hour urinary collecting period a significant diurnal variation in AER was found in the 33 patients, with the lowest values at night ($P < 0.001$). In addition, a significant difference in SI was seen with the highest values at night ($P = 0.01$, Table 2). Furthermore, a negative correlation between SI and AER was found in each of the urinary collecting periods: $r = -0.54$, $P = 0.001$ (period A), $r = -0.75$, $P < 0.00001$ (period B) and $r = -0.34$, $P = 0.06$ (period C). When only looking at group D_0 similar results were obtained (Table 2). Within this group U_{RBP} was measured in 16 patients. In these patients U_{RBP} remained stable comparing the different sampling periods during the 24 hours: period A, 69.5 (37 to 530) μg/liter (median, range); period B, 71.5 (37 to 520) μg/liter; and period C, 78.0 (33 to 1640) μg/liter ($P = 0.09$). Although no diurnal difference in U_{RBP} was found in this subgroup of patients a significant difference in SI ($P = 0.02$) and AER ($P < 0.001$) was maintained.

Average 24 hour BPs during day and night and Δ-BP are given in Table 2. The patients were mainly normotensive and blood

Table 2. Diurnal variation in AER, SI and blood pressure in the total study population and in the subgroups D_0 and D_A

		N	Period A (8.00 a.m.–12.00 a.m.)	Period B (12.00 p.m.–bedtime)	Period C (bedtime–8.00 a.m.)	P value
AER[a] *μg/min*	Total	33	10 (33–137)	8 (3–84)	5 (0–78)	<0.001
	D_0	27	7 (3–29)	6 (3–21)	5 (0–18)	<0.001
	D_A	6	95 (23–137)	68 (40–84)	46 (14–78)	0.03
SI	Total	33	1.6 (0.2–3.8)	1.7 (0.3–3.0)	2.0 (0.2–4.0)	0.01
	D_0	27	1.8 (0.7–3.8)	1.9 (0.8–3.0)	2.2 (0.7–4.0)	0.002
	D_A	6	1.0 (0.2–1.9)	0.7 (0.3–1.0)	1.0 (0.2–1.7)	0.85
sBP *mm Hg*	Total	33	133 (115–161)		116 (97–153)	<0.001
dBP *mm Hg*	Total	33	83 (72–101)		68 (54–90)	<0.001
ΔsBP (%)	Total	33	12 (−9–39)			
ΔdBP (%)	Total	33	18 (3–34)			

Data are presented as mean (range).
[a] median (range).
ΔBP is the percentage change from day to night, (average daytime-BP − average nighttime-BP)/(average daytime-BP) × 100.

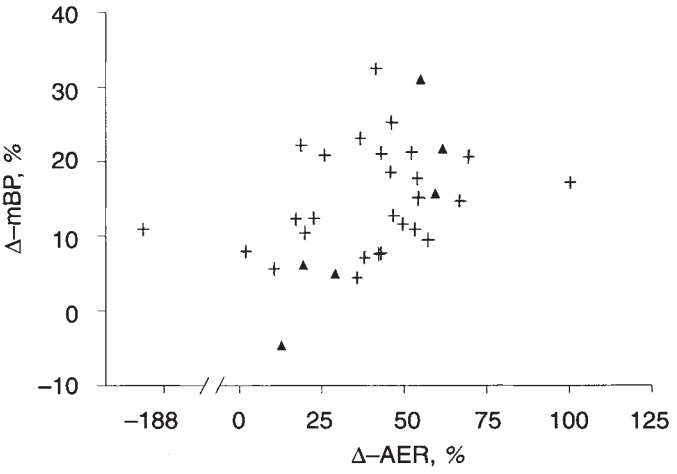

Fig. 1. *Correlation between diurnal variation in blood pressure (Δ-mBP) and diurnal variation in urinary albumin excretion rate (Δ-AER).* The correlation was significant in patients with incipient nephropathy (group D_A, ▲; $r = 0.87$, $P = 0.02$). In normoalbuminuric patients (group D_0, +) no significant correlation was found ($r = 0.20$, $P = 0.32$).

pressures in groups D_0 and D_A were comparable. No correlation between Δ-BP and Δ-AER was found neither in the combined population or in group D_0 separately (Fig. 1). However, in group D_A there was a significant positive correlation between these two parameters ($r = 0.87$, $P = 0.02$, Fig. 1).

One patient, belonging to group D_0, had an unexplained nightly excretion of albumin vastly greater than the daily loss. Excluding this patient from the study did not alter the results.

Discussion

The present study confirms previous results showing a significant negative correlation between AER and SI in patients with type 1 diabetes [12] In addition to the earlier study the present data demonstrate that even within 24 hours, a diurnal variation of AER seems to be associated to a diurnal variation in SI.

What are the physiological mechanism causing this diurnal variation in SI? It has been shown that changes in renal blood flow alter the fractional distribution of blood flow between the inner and outer cortex [15]. Furthermore, it has been suggested, that qualitative differences exist in permeability between the glomeruli in the inner and outer renal cortex [16]. Thus, an interglomerular unequal distribution of anionic molecules could explain that a diurnal variation in SI might be influenced by a shift in the population of perfused glomeruli. Another explanation for a diurnal variation in SI could be physiological day and night pressure alterations within the kidney tissue, that is, pressure reduction during nighttime might influence the morphology of the glomerular basement membrane. At a relaxed stage the basement membrane may contract, thus presenting an increasing amount of anionic charge (HSPG) per area filtration surface. During night this could account for the concomitant reduction in AER and in increment in SI, demonstrated in the present study.

Altered tubular charge may influence the excretion level of urinary albumin, -IgG, and -IgG_4. Although we were only able to measure U_{RBP} in 16 out of 27 normoalbuminuric diabetic patients, we found no diurnal variation in U_{RBP}. Unaltered diurnal U_{RBP} in normoalbuminuric patients with IDDM has recently been confirmed by others [17]. Furthermore, earlier studies have demonstrated that in patients with IDDM, tubular function is well preserved until advanced diabetic nephropathy occurs [7, 18]. Thus, in our group of patients it is unlikely that altered tubular charge influence our results.

It was emphasized that during the 24-hour study period the patients should continue their ordinary daily activities, and thus blood samples for S_{IgG} and S_{IgG4} were not taken during the urine sampling periods. The SI calculations are therefore all made on the basis of morning S_{IgG} and S_{IgG4} levels. Only few studies concerning diurnal variations of S_{IgG} and S_{IgG4} have been published. In patients with rheumatoid arthritis circulating immune complexes showed a circadian variation presumably related to variations in subjective clinical assessment of illness [19]. Except for having diabetes all our patients were healthy and we did not find any significant day-to-day variation in S_{IgG} and S_{IgG4} levels. We find no reasons to believe that a diurnal variation of the S_{IgG} and S_{IgG4} levels should be of major importance for our results.

Due to a U_{IgG4} excretion below the detection level of our assay, twelve patients (11 being normoalbuminuric and 1 with incipient nephropathy) were excluded from the study. Whether this loss of patients influences the results remains unknown. Meanwhile, in five out of the twelve patients (4 being normoalbuminuric and 1 having incipient nephropathy) U_{IgG4} measurements were only missing from the nighttime period. Including these patients in the study ($N = 38$), estimating the missing U_{IgG4} level to 8 μg/liter (the detection level of the assay) corresponding to the lowest possible nighttime SI, the diurnal variation in SI remained significant with the highest values at night ($P = 0.03$), supporting our presented data.

The positive correlation between diurnal variation in blood pressure and AER among the six patients with albuminuria has recently been confirmed by others [17]. The prognostic implications of abnormal diurnal variations in blood pressure are not fully established yet, but it could be an additional marker for the

progression of albuminuria. Thus, diurnal variation in AER in patients with incipient nephropathy seems to be associated with diurnal variations in blood pressure and not to or only to a minor degree to variations in SI. Whether diurnal variation in blood glucose influence this result remains unanswered.

We suggest that in normoalbuminuric patients with IDDM, the diurnal variation in 24 hour AER is related to a diurnal variations in SI and not to variations in BP.

Reprint requests to Dr. Pernille Mørk Hansen, Steno Diabetes Center, Niels Steensensvej 2, DK-2820 Gentofte, Denmark.

References

1. KOFOED-ENEVOLDSEN A, BORCH-JOHNSEN K, KREINER S, NERUP J, DECKERT T: Declining incidence of persistent proteinuria in type 1 (insulin-dependent) diabetic patients in Denmark. *Diabetes* 36:205–209, 1987
2. TAMSMA JT, VAN DEN BORN J, BRUIJN JA, ASSMANN KJM, WEENING JJ, BERDEN JHM, WIESLANDER J, SCHRAMA E, HERMANS J, VEERKAMP JH, LEMKES HHPJ, VAN DER WOUDE FJ: Expression of glomerular extracellular matrix components in human diabetic nephropathy: Decrease of heparan sulphate in the glomerular basement membrane. *Diabetologia* 37:313–320, 1994
3. VAN DEN BORN J, VAN DEN HEUVEL LPWJ, BAKKER MAH, VEERKAMO JH, ASSMANN KJM, BERDEN JHM: A monoclonal antibody against GBM heparan sulfate induces an acute selective proteinuria in rats. *Kidney Int* 41:115–123, 1992
4. PARTHASARATHY N, SPIRO RG: Effect of diabetes on the glycosaminoglycan component of the human glomerular basement membrane. *Diabetes* 31:738–740, 1982
5. VERNIER RL, STEFFES MW, SISSON-ROSS S, MAUER M: Heparan sulfate proteoglycan in the glomerular basement membrane in type 1 diabetes mellitus. *Kidney Int* 41:1070–1080, 1992
6. MAKINO H, IKEDA S, HARAMOTO T, OTA Z: Heparan sulfate proteoglycans are lost in patients with diabetic nephropathy. *Nephron* 61:415–421, 1992
7. DECKERT T, FELDT-RASMUSSEN B, DJURUP R, DECKERT M: Glomerular size and charge selectivity in insulin-dependent diabetes mellitus. *Kidney Int* 33:100–106, 1988
8. DECKERT T, KOFOED-ENEVOLDSEN A, VIDAL P, NØRGAARD K, ANDREASEN HB, FELDT-RASMUSSEN B: Size- and charge selectivity of glomerular filtration in Type 1 (insulin-dependent) diabetic patients with and without albuminuria. *Diabetologia* 36:244–251, 1993
9. KOFOED-ENEVOLDSEN A, JENSEN K, DECKERT T: Glomerular charge selectivity in normoalbuminuric type 1 diabetic patients. (abstract) *Diabetologia* 35(Suppl 1):A144, 1992
10. BANGSTAD H-J, KOFOED-ENEVOLDSEN A, DAHL-JØRGENSEN K, HANSSEN KF: Glomerular charge selectivity and the influence of improved blood glucose in Type 1 (insulin-dependent) diabetic patients with microalbuminuria. *Diabetologia* 35:1165–1169, 1992
11. BANGSTAD H-J, ØSTERBY R, DAHL-JØRGENSEN K, BERG KJ, HARTMANN A, HANSSEN KF: Improvement of blood glucose control in IDDM patients retards the progression of morphological changes in early diabetic nephropathy. *Diabetologia* 37:483–490, 1994
12. HANSEN PM, MATHIESEN ER, KOFOED-ENEVOLDSEN A, DECKERT T: Possible effect of angiotensin converting enzyme inhibition on glomerular charge selectivity. *J Diab Comp* 9:158–162, 1995
13. KOFOED-ENEVOLDSEN A, JENSEN K: Measuring urinary IgG and IgG_4 excretion. (abstract) *Clin Chem* 37:1136, 1991
14. JENSEN T, DECKERT M, DAWNAY A, FELDT-RASMUSSEN B: Micro-ELISA for the quantitation of human urinary and serum retinol-binding protein. *Diabetes Res* 10:93–95, 1989
15. ITSKOVITZ HD, TERRAGNO NA, MCGIFF JC: Effect of a renal prostaglandin on distribution of blood flow in the isolated canine kidney. *Circ Res* 34:770–776, 1974
16. ARISZ L, DONKER AJM, BRENTJENS JRH, VAN DER HEM GK: The effect of indomethacin on proteinuria and kidney function in the nephrotic syndrome. *Acta Med Scand* 199:121–125, 1976
17. PONTUCH P, VOZAR J, TOSEROVA E, BULAS J, KRATOCHVILOVA H: Albuminuria, retinol-binding protein excretion and ambulatory blood pressure in type l diabetes. (abstract) *Diabetologia* 37(Suppl 1):A194, 1994
18. STEHOUWER CDA, FISCHER HRA, HACKENG WHL, OTTOLANDER GJH, DONKER AJM: Diurnal variation in urinary protein excretion in diabetic nephropathy. *Nephrol Dial Transplant* 6:238–243, 1991
19. PETERSEN I, BAATRUP G, BRANDSLUND I, TEISNER B, RASMUSSEN GG, SVEHAG SE: Circadian and diurnal variation of circulating immune complexes, complement-mediated solubilization, and the complement split product C3 days in rheumatoid arthritis. *Scand J Rheumatol* 15:113–118, 1986